Olfactory Reversal Learning in BALB/c Inbred Mice

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Until recently the role of olfaction in species-typical and learned behavior had received little attention from behavioral scientists. In the past few years, an increasing number of studies have demonstrated that odors provide salient cues for guiding behavior and that several macrosmatic species may be dependent upon the integrity of the olfactory system for performance of essential biologic activities (Eisenberg & Kleiman, 1972). Since a number of olfactometers which provides precise control of the odor stimulus and environment have been described for human and animal studies (e.g., Pfaffman, Goff, & Bare, 1958; Henton, Smith, & Tucker, 1966; Braun, Wermuth, & Harberly, 1967; Moulton, Celebi, & Fink, 1970; Stone, Pryor, & Steinmertz, 1969; Williams & Slotnick, 1970; Kaneko, 1975), many studies concerning olfactory discrimination learning have been done. In the most of these studies rats have been served as a subject to demonstrate that macrosmatic animals such as rodents might perform differently with olfactory cues than with the auditory and visual stimuli commonly used in the discrimination learning. There is some reason to expect that the performance of mice in olfactory reversal learning might differ from rats. Mice behave passively in a new situation, while rats are active. The difference can result in a different performance on a reversal learning. In a recent systematic study, learning set formation of rats has shown that the animals showed a dramatic decrease in errors over a series of reversals, and asymptotic performance approached nearly errorless learning (Nigrosh, Slotnick, & Nevin, 1975).

The present experiment provides a test to show the difference of performance between rats and mice in the olfactory reversal learning. Also this experiment was designed to investigate the relatiohship between the learning type and performance by using BALB/c inbred mice which includes mutant of absence of curpus corsum.

METHOD

Subjects

Four BALB/c and inbred male mice served as subjects. All animals were

raised in our laboratory, weaned at 21 days of age and housed as same-sex littermates in a temperature controlled vivarium. In both rearing and experimental conditions, the subjects were exposed to a 12/12 light and dark cycle, with the light period beggining at 0800 each morning. A week before the biggining of the experiment, the subjects were housed individually and kept on a 23 hr water-deprivation schedule. Food was available ad lib throughout the experiment. On the day of the experiment, the subjects ranged from 56 to 60 days of age.

Apparatus

An apparatus consisted of a test chamber and odorant delivery system. Odor stimuli were generated and controlled by an odorant delivery system, diagrammed in Figure 1. The output of an air compressor, controlled by a pressure regulator, was filtered through separate columns of Drierite ($CaSO_4$) and charcoal. The dehumidified and deodorized air was divided into a carrier stream and two independent odorant streams. Air in the carrier stream was led continuously into the test chamber via a 2 cm diameter manifold at a rate of 10 liters/min. Odorant streams were independently passed over the surface of 25 cc of liquid odorant contained in 500 cc gas washing bottles at a rate of 10 cc/min. Odorized air flow from each gas washing bottle was collected directly to the common port of a 3-way Teflon electric solenoid valve. The normally open port of each valve was connected to an exhaust pathway, and the normally closed port was connected to the manifold to which the carrier stream was connected. The odorized air traveled 45 cm from the normally

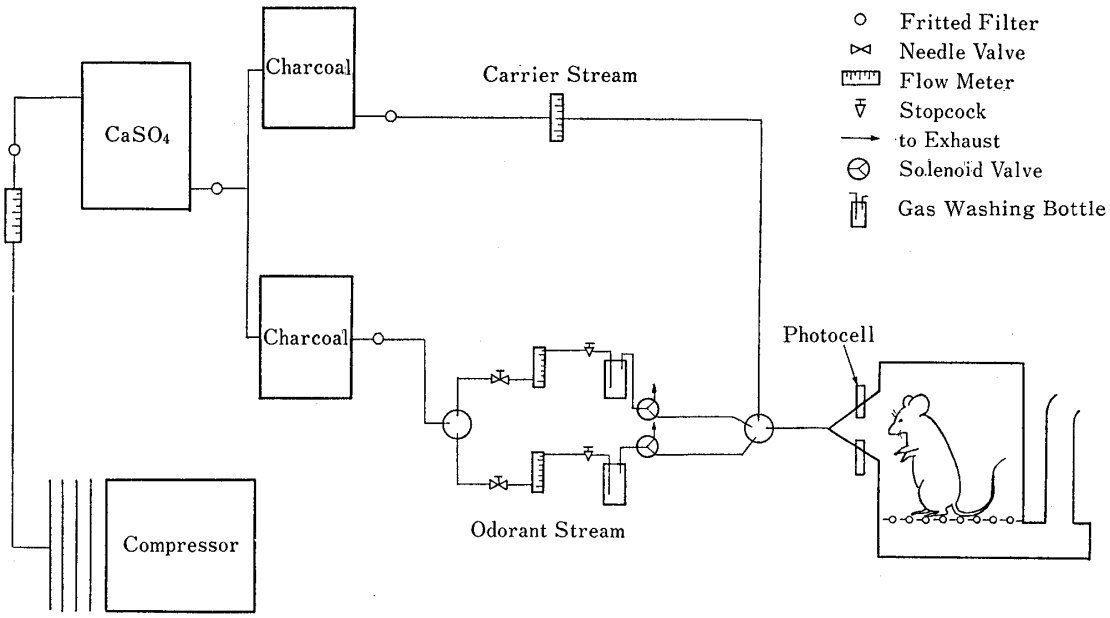

Fig. 1 Olfactometer

closed port of each odor control valve to the test chamber through 4 mm (inside diameter) glass tubing at a velocity of approximately 50 cm/sec. The odor delivery system was made of glass or Teflon.

The test chamber (15×12×15 cm) was constructed of clear Plexiglas and stainless steel in a manner which permitted regular and frequent washing by total immersion procedures. On side of the test chamber was a stainless steel panel on which a modified 3 cm diameter glass funnel was located in the center, a response lever was located 1 cm from the center to the left, and an 8 cm diameter glass cup was located at the center bottom. A photobeam was positioned across the neck of the funnel approximately 1 cm outside of the test chamber panel. The response lever was a 3 mm diameter stainless steel ball and required approximately 1 g operating force to detect lever pressing by a photocoupler. Water reinforcement was delivered to the glass cup through a polyethylene tubing by a syringe pump. Air from the chamber was continuously exhausted to the outside of the building by a miniture fan connected to the bottom of the chamber by a 3 cm diameter flexible plastic hose.

Procedure

Mice were restricted to 2 cc of water per day for 7 days prior to training. This water deprivation was maintained throughout the experiment. Water was withheld in the home cage of each mouse until the mouse was able to earn 2 cc of water during an 1 hour daily shaping session. The body weight of each mouse was maintained at approximately 80% of the weight prior to deprivation by this schedule.

Initially mice were trained to lever press on a continuous 0.02 cc water reinforcement schedule. After mice were able to earn 2 cc of water within 30 min, responses were then reinforced only when they were made while the funnel was illuminated directly by a miniture light bulb. The funnel was illuminated for 6 sec at variable intervals averaging 10 sec. When responses occured reliably during the illuminated trial, non-illuminated trials and the continuous carrier air stream were intruduced, and in further trials illumination and electric valve were generated contingent upon the mouse breaking the photobeam at the funnel. In each session 100 illuminated and 100 non-illuminated trials were presented in a random order. The intertrial interval was fixed at 3 sec. When the intertrial responses or the responses in non-illuminated trials occurred, the last response was followed by a 3 sec delay before the next trial could be initiated. After the acquisition of this response sequence, the initiation of the illumination was gradually postponed to 1 sec after the photobeam was broken, and the duration of the illumination was decreased to 2 sec. When the mouse reached the criterion of responding correctly to 90% of the total responses,

odor discrimination training began in the next session. Iso-amyle acetate odor (S^+) was intruduced during the illuminated trials and ethyle acetate odor (S^-) was intruduced during the non-illuminated trials. The valves controlled the stimuli were operated for 3 sec at the beginning of each trial, and responses were effective for 6 sec after the valve was operated. The illumination was then turned off after mice completed 2 sessions. The reinforcement schedule was gradually changed to fixed-ratio 5 sec. Sessions consisted of 100 trials per day until a criterion of responding correctly to 80% in 20 consecutive trials. Training was terminated when criterion performance was achived and a series of six discrimination reversal problems were given 24 hr later. In the reversal session, the reinforcement values of the S^+ and S^- stimuli were reversed, and traning was continued until criterion was reached.

RESULTS

Percent correct resposes on the acquisition training with light are illustrated in Figure 2.

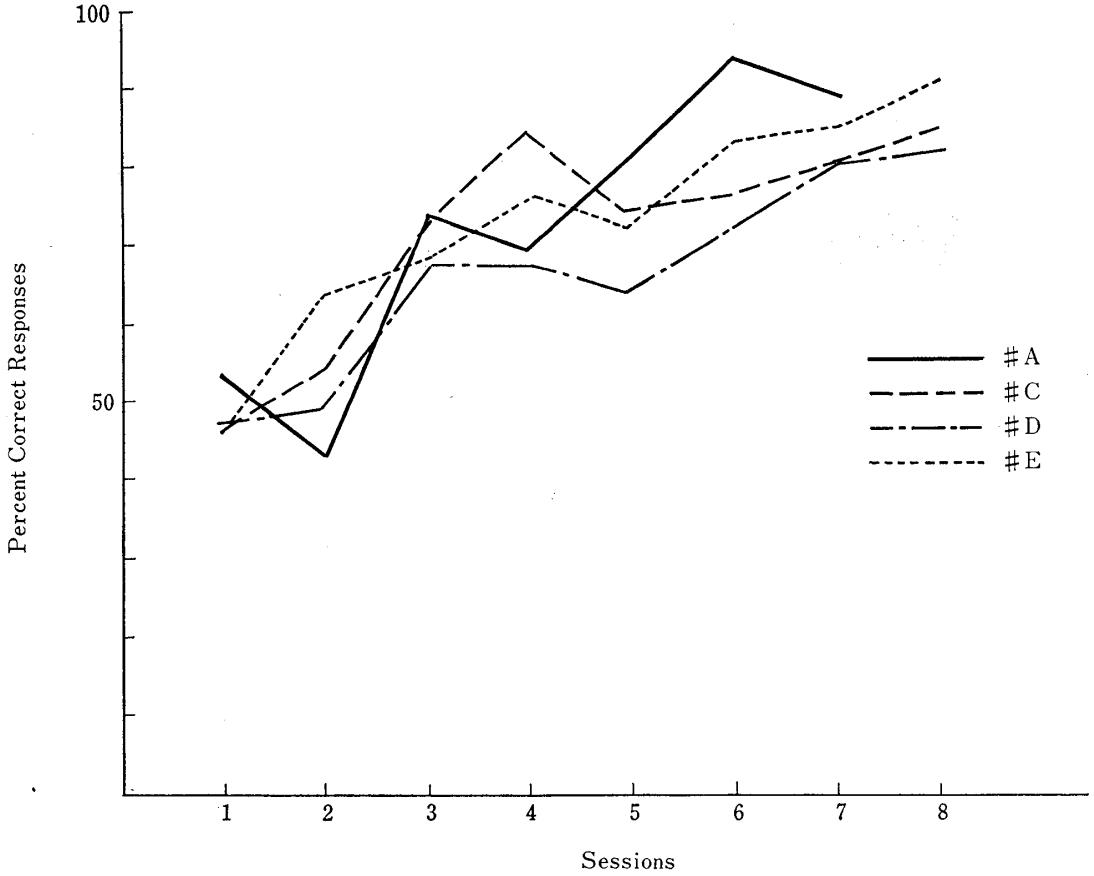

Fig. 2 Percent correct responses in the acquisition training with light.

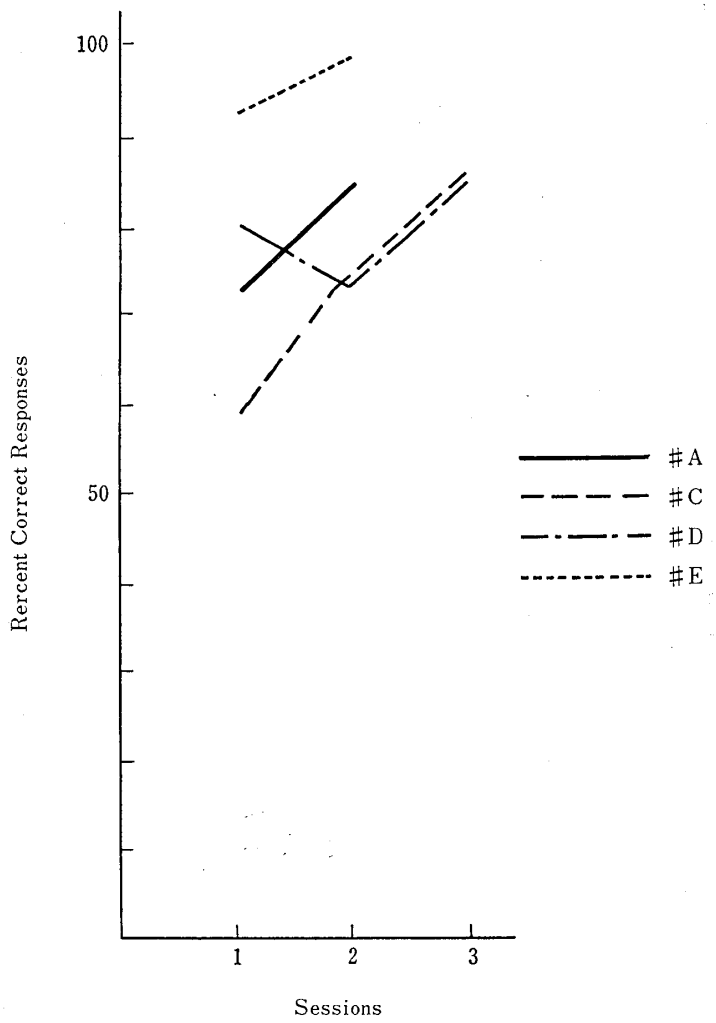

Fig. 3 Percent correct responses during odor discrimination training with light.

All animals show criterion discrimination performance for light stimulus within 8 sessions. Figure 3 shows the rate of correct responses during odor discrimination training with light. Each animal except #E made more errors in the acquisition training than in odor discrimination training with light.

Error scores for serial reversal training are illustrated in Figure 4. Two animals, #D and #E, made only a few errors on the original learning. On the other hand #A made near to handred errors in odor discrimination training after the light turned off. These three animals showed negative transfer on the first reversal.

The error scores for the first reversal were almost 200 times in evry animals. On second reversal, #D and #E showed improvement in the acquisition. #A showed rapid acquisition in the second reversal. #C made over 350 errors on the original learning.

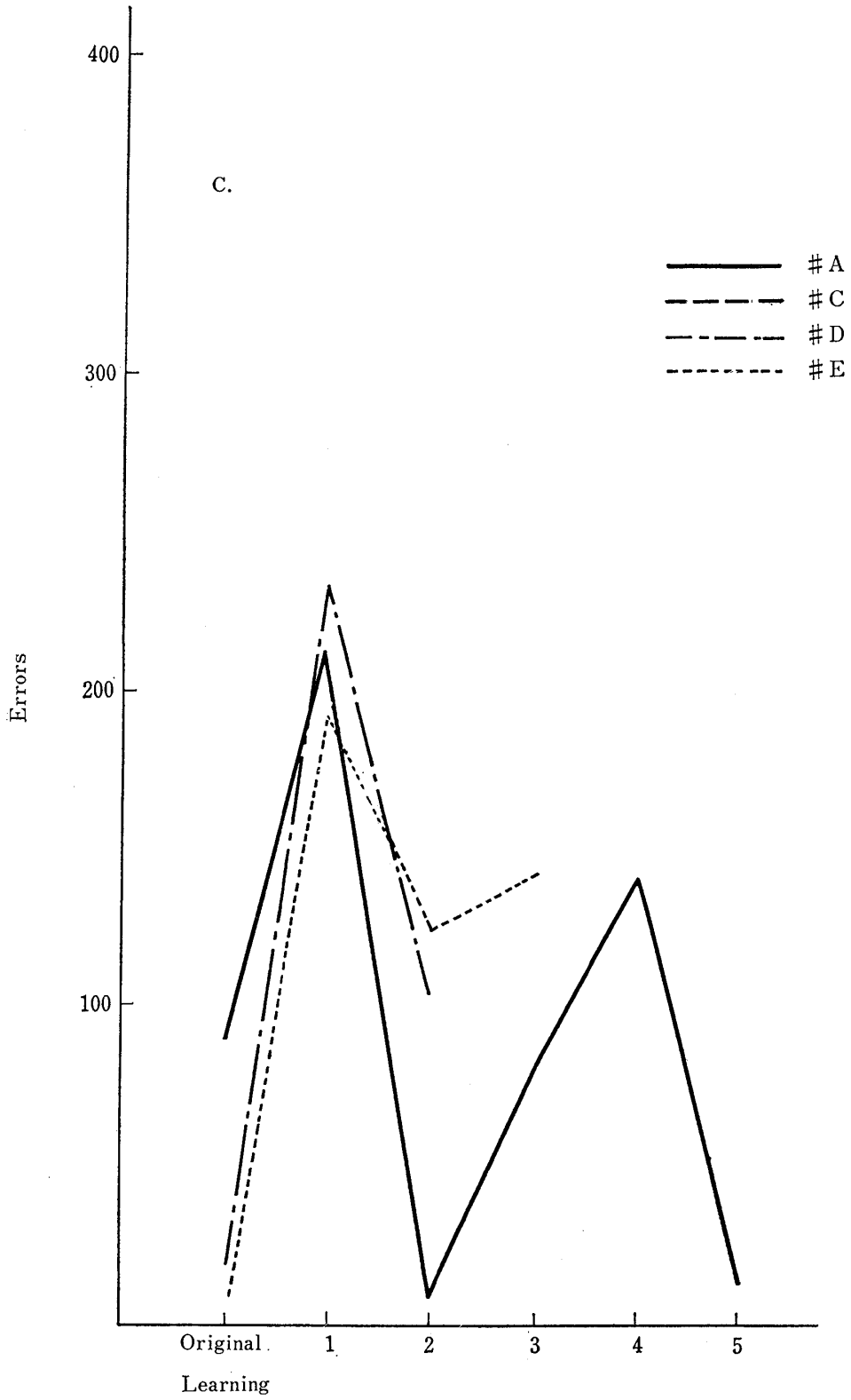

Fig. 4 Number of errors on original learning and successive odor discrimination reversals.

Analysis of individual acquisition functions revealed that the animal responded to the both S^+ and S^- stimuli in the first reversal reached criterion faster than the animal which did not response to the both stimuli. The responding to the both stimuli resulted in the rapid acquisition of second reversal in #A. The subjects #D and #E did not respond to the both stimuli at the first part of evry reversal trials.

For each mouse, behavior on the first reversal was characterized by two patterns. While the behavior of the subject #A was characterized by a period of responding to both S^+ and S^- followed by a gradual decrease in S^- responding, the behavioral pattern of #D and #E was characterized by a period of no responding to both S^+ and S^- followed by a gradual increase in both S^+ and S^- responding.

DISCUSSION

Previous study in rats indicated that successive discrimination reversal learning depended on stimulus modality (Nigrosh & Slotnick). All rats trained by odor showed positive transfer on the first reversal and asymptotic performance of only a few errors in later reversals. In this experiment, the subject gave data typical of that reported for vidual discriminations, with an increase in errors on the first reversal, followed by systematic improvement (e.g., Gonzalez, Berger, & Bitterman, 1966; Gonzalez, Roberts, & Bitterman, 1964). Thus, the choice of animal may be as important as the choice of stimulus. Also, this experiment suggested that the behavior type characterized by responding to the stimuli in novel situation is consistently correlated with improvement in later reversals. By using BALB/c inbred mice which have inherited deficit of the absense of corpus callosum, two types have been demonstrated in the training session. It is necessary to analize the errors made in acquisition training. Rats may respond more often to stimuli than normal mice may do.

Mackintosh (1969) has argued that improvement in successive reversals depends in part on the ability to attend to stimuli that are not consistently correlated with reinforcement. The performance on odor discrimination learning without light is clearly consistent with his views. Some of the BALB/c mice showed virtually complete generalization to all test stimuli in odor generalization test (Kaneko, 1980). It could be that some BALB/c mice do not have the ability to attend to odor stimuli.

A major contribution of the present results is the demonstration that one such variable, animal type, may play a critical role in the form of the learning function.

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